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Field Leaf-Test Kit for Rapid Determination of Dislodgeable Foliar Residues of Organophosphate and N-Methyl Carbamate Insecticides

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Measurement of dislodgeable foliar residues (DFR) has served two important functions; 1. the estimation of dissipation rates for pesticides from leaf surfaces, and 2. the establishment of residue levels for worker reentry into fields treated with acetylcholinesterase- (AChE) inhibiting pesticides (Knaak 1980; Knaak and Iwata 1982; Maddy 1986). Substantial work in California is currently being performed to evaluate the feasibility of using DFR in establishing safe work levels to guide reentry into pesticide-treated crops.

Current foliar sampling for determination of DFR has generally been adapted from the work of Gunther et al. (1973), and Iwata et al. (1977). A mechanical leaf punch is used to remove a leaf disk of known size and area (10 cm^2, both surfaces). Leaf disks are transported on ice to a laboratory for extraction in an aqueous-surfactant solution. Secondary extraction of the analyte into an organic solvent is performed prior to analysis by gas-liquid chromatography (glc) or other suitable procedure. Results are reported in ug pesticide per cm^2 leaf. Laboratory analysis entails sample transport, use of organic solvents for residue extraction, the expenses of a skilled technician and necessary analytical instrumentation, and often a time delay (24 hr or more) between taking samples and obtaining results.

Recently a field leaf-test kit[1] has been developed that measures AChE inhibition activity from foliar residues of organophosphorus (OP) and N-methyl carbamate pesticides. Based on the level of inhibition present and the kit's detection limit, a semiquantitative estimate of DFR (ug/cm^2) can be obtained. These studies were performed to compare DFRs obtained by traditional solvent extraction and instrumental analysis with results of the rapid field test kit.

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MATERIALS AND METHODS

Leaves from grapes, pears, almonds and pumpkins which had been treated with phosalone, chlorpyrifos, azinphos-methyl, methomyl, or phosmet were sampled using a 2.5-cm diameter leaf-punch. Forty leaf punches were placed in a 4-oz glass jar. The jar was sealed with aluminum foil and capped. Duplicate sets of samples were taken from the same field area and stored on ice. One set was shipped to the California Department of Food and Agriculture's (CDFA) laboratory in Sacramento and the other set to the test kit manufacturer. Results from the kit analysis were sent to CDFA for comparison to our laboratory's glc results. One set of phosalone treated grape leaves (CB series) and one set of methomyl treated pumpkin leaves were also analyzed in the field by CDFA personnel using the test kit with duplicate samples sent for glc analysis.

Upon arrival at the CDFA laboratory four drops of a 2% Sur-Ten (dioctyl sodium sulfosuccinate) surfactant solution and 50 mL of water were added to the sample jars, and the jars were rotated for 20 min. The wash process was repeated twice for each sample. The wash solutions were decanted, combined and mixed with ethyl acetate (dichloromethane for methomyl extraction) in a separatory funnel; the ethyl acetate layer was decanted through anhydrous sodium sulfate. The decanted solution was concentrated or diluted as necessary for analysis by gas-liquid chromatography.

The test kit contained an aqueous-surfactant extraction solution, bromine reagent, color-coded reagent tubes, plastic graduated cylinder and a plastic squeeze dropper. A measured volume (10-20 mL) of the aqueous-surfactant extraction solution was added to the leaf samples, and the mixture was agitated for five min. The bromine reagent (solution or tablet) was then added to the extraction supernatant to oxidize the parent OP compound to its oxon. A one-mL aliquot of the supernatant was placed into a marked tube containing the buffer and color reagent and gently agitated. The solution was then added and mixed with the AChE in the second tube. Controls using one-mL aliquots of the extraction solution were simultaneously prepared. Acetylcholinesterase inhibition activity was indicated by a lack of color formation. If there was no or minimal AChE inhibition activity a deep blue color developed in five to 10 min. Serial dilutions of leaf extraction solution were made until only a minimal amount of cholinesterase inhibition activity could be detected, which represented the minimum detection level (MDL) for that pesticide. The following equation was used to calculate the amount of residue present:

$$\text{ug/cm}^2 = \frac{\text{MDL}^a \text{ (ppm) X Dilution Factor}^b}{\text{Surface Area (cm}^2\text{)}}$$

MDL^a = Minimum Detection Level in ppm for the specific pesticide. Typical detection levels are 1-5 ppm (manufacturer's data).

Dilution Factor[b] = the volume of extraction solution and the number of dilutions made, i.e., if 10 mL of extraction solution were used, the dilution factor = 10 for an undiluted sample. If a 1:1 dilution of the extraction solution were made the Dilution Factor would be 20.

RESULTS AND DISCUSSION

A comparison was made between a field ready kit and laboratory glc methods to estimate foliar organophosphate and carbamate pesticide residues. The two methods were in good agreement, with the AChE inhibition field kit results within ± 0.1 ug/cm^2 of the glc methodology (Table 1). The comparability of the methods was supported by a regression of the AChE and glc data sets: AChE = 0.97(glc) + 0.005, r = 0.97. ELISA and glc methodologies were similarly compared by Hammock and Mumma (1980). Independent use of the test kit by CDFA personnel (CB grape and pumpkin samples, Table 1) also provided residue estimations in agreement with glc data. Using these DFRs the exposure potential of the pesticides can be estimated. For example, the phosalone level on grapes of the CB series was about 1 ug/cm^2. We have recently estimated the transfer factor (TF) for wine grape harvesters to be 20,000-95,000 cm^2/hr (*Krieger et al., unpublished*). The estimated potential hourly (external) exposure (DFR x TF) is 95 mg/hr. This rate of exposure would be 10-times the rate estimated for the phosalone-treated S50B series grapes (95,000 cm^2/hr x 0.1 ug/cm^2, Table 1). The estimated safe work level for persons harvesting phosalone-treated grapes is about 50 mg/hr (*Krieger et al., unpublished*). On this basis, harvesting or other high foliage-contact work in CB grapes would not be permitted due to excessive exposure potential. Recent California regulations have increased the reentry interval on grapes treated after July 1 with methomyl to 21 days or if the DFR is ≤ 0.1 ug/cm^2. The viticulturalist could use this kit to estimate the DFR. When DFR approaches 0.1 ug/cm^2, leaf samples would be taken to a laboratory for chemical analysis prior to worker reentry.

Occasionally serial dilutions of the extraction solution did not result in a clear endpoint, as in the phosalone pear data (Table 1). A range in values was presented in these instances. Clarification of the endpoint can be achieved by adjusting the amount of leaf area extracted, volume of extraction solution used, or by using less than a full 10-fold dilution. Strong inhibition activity resulted in no color change while weak to no activity resulted in graded levels of blue within a few min. To adequately gauge the rate and intensity of a color change it was helpful to prepare controls before and after preparation of the unknown samples.

Laboratory analysis of foliar dislodgeable residue samples generally requires 24-48 hr or longer for results to be obtained. Rapid techniques for OP determination have been reported but still require a laboratory setting for analysis (Ragab 1967). In contrast, four samples were analyzed in the field by relatively

Table 1. Determination of dislodgeable residue levels by gas-liquid chromatography and cholinesterase inhibition activity.

Foliage	Sample	Pesticide	GLC	AChE Inhibition
			-------- ug cm^{-2} --------	
Grape	S51B	Phosalone	0.19	0.1
	S52B		0.41	0.1
	S53B		0.09	0.0
	S54B		0.01	0.0
	S55B		0.06	0.0
		Mean ± (SE)	0.15(0.07)	0.1(0.02)
Grape	CB160	Phosalone	0.95	0.9
	CB165		0.95	0.8
	CB175		0.89	0.8
	CB180		0.50	0.8
		Mean ± (SE)	0.82(0.11)	0.8(0.02)
Pear	SS83A	Phosalone	1.38	1.0-2.0
	SS85A		1.55	1.0-2.0
	SS87A		1.38	1.0-2.0
	SS89A		1.42	1.0-2.0
	SS91A		1.71	1.0-2.0
		Mean ± (SE)	1.49(0.06)	1.5
Almond	CB14D34A	Chlorpyrifos	ND[1]	0.0
Almond	CB14D161A	Azinphos-methyl	0.105	0.1-0.2
	CB14D163A		0.009	0.0
		Mean ± (SE)	0.057(0.048)	0.1
Almond	CB15D34A	Phosmet	1.50	0.8-1.9
	CB15D151A		0.97	0.8
	CB15D153A		0.54	0.8
		Mean ± (SE)	1.00(0.28)	1.0
Pumpkin	CBMED1	Methomyl	0.10	0.2
	CBMED2		0.09	0.1
	CBMED3		0.06	0.1
		Mean ± (SE)	0.08(0.01)	0.1(0.03)

[1] ND = None Detected

inexperienced personnel using the leaf-test kit including serial dilutions and controls within 45 min after foliar sampling. Calculation of the residue per cm^2 leaf area is straightforward providing the user with a rapid estimate of foliar residue.

The leaf-test kit has the advantages of being rapid, not requiring special skills, organic solvents or instrumentation, and is relatively inexpensive to use ($2-$3/sample). The leaf-test kit differs in the minimum detectable limits for each pesticide, and since it relies on visual observation of color changes is semiquantitative. The kit also requires the addition of a bromine reagent to oxidize the parent OP compound to its oxon derivative. Thus, separate analysis for the parent compound and the oxon although possible is more tedious. Analysis for N-methyl carbamates does not require bromine activation. The kit can be used to check fields qualitatively for the presence of AChE inhibitors. For estimation of field residue levels the pesticide applied and the kit's minimum detection level for that pesticide must be known. Detection levels lower than 0.1 ug/cm^2 may be possible for some pesticides by manipulation of the leaf surface area and extraction solution volume.

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REFERENCES

Gunther FA, Westlake WE, Barkley JH, Winterlin W, Langbehn L (1973) Establishing Dislodgeable Pesticide Residues on Leaf Surfaces. Bull Environ Contam Toxicol 9:243-249.

Hammock BD, Mumma RO (1980) Potential of Immunochemical Technology for Pesticide Analysis. ACS Symp 136:321-352.

Iwata Y, Knaak JB, Spear RC, Foster RJ (1977) Worker Reentry into Pesticide Treated Crops. I. Procedure for the Determination of Dislodgeable Pesticide Residues on Foliage. Bull Environ Contam Toxicol 18:649-655.

Knaak JB (1980) Minimizing Occupational Exposure to Pesticides: Techniques for Establishing Safe Levels of Foliar Residues. Residue Rev 75:81-96.

Knaak JB, Iwata Y (1982) The Safe Level Concept and the Rapid Field Method: A New Approach to Solving the Reentry Problem. In: Plimmer JR (ed) Pesticide Residues and Exposure. ACS Symposium Series 182. American Chemical Society, Washington, D.C. pp 23-39.

Maddy KT (1986) Estimated Safe Levels of Foliar Pesticide Residues to Allow Unprotected Workers Reentry into Treated Fields in California. HS-1280. California Department of Food and Agriculture. Sacramento, California.

Ragab MTH (1967) 4-(p-Nitrobenzyl)Pyridine as a Spray Reagent for Organophosphorus Pesticides and some of their Breakdown Products on Thin-layer Chromatograms. Bull Environ Contam Toxicol 2:5:279-288.

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